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Description of the new species *Calcarobiotus* (*C.*) *longinoi* sp. nov. (Eutardigrada, Macrobiotidae) from Costa Rica with the diagnostic key to the genus *Calcarobiotus*

ŁUKASZ KACZMAREK[1], ŁUKASZ MICHALCZYK[2] & ROBERTO GUIDETTI[3]

[1]*Department of Animal Taxonomy and Ecology, A. Mickiewicz University, Poland,* [2]*Centre for Ecology, Evolution and Conservation, School of Biological Sciences, University of East Anglia, UK, and* [3]*Department of Animal Biology, University of Modena and Reggio Emilia, Italy*

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Abstract
A new species of Tardigrada (Eutardigrada), *Calcarobiotus longinoi* sp. nov., is described from moss samples collected in Alajuela Province (Costa Rica). The presence of genus *Calcarobiotus* is new for Central America. This species differs from other species of the genus *Calcarobiotus* by lacking of spurs on the basal portion of the claws. On the base of this new finding, the diagnosis of the subgenus *Calcarobiotus* is emended. A taxonomic key to the genus *Calcarobiotus* is also presented.

Keywords: *Tardigrada,* Calcarobiotus longinoi *sp. nov., new species, distribution, taxonomy, SEM observations*

Introduction

The genus *Calcarobiotus* was described by Dastych in 1993 based on two African species: *Calcarobiotus filmeri* Dastych, 1993 and *C. occultus* Dastych, 1993. Dastych (1993) identified this genus within Macrobiotidae on the basis of the presence of spurs on the basal portion of claws. In 2001, Guidetti and Bertolani emended the genus diagnosis and gave the definition of the claws of *Calcarobiotus* type (wide basal portion of the claw with large distal part, bearing or no spurs; wide transverse septum between basal portion and rest of the claw; main and secondary branches similar and almost symmetrical with respect to the median plan of the claw). They divided the genus *Calcarobiotus* into two subgenera: the nominal subgenus *Calcarobiotus* (claws with similar shape on all legs; basal portion with one or two lateral spurs) and the new *Discrepunguis* (hind claws of different shape with respect to those on the first three pairs of legs, showing a small basal portion, long common tract and short secondary branches). In this paper a new species of *Calcarobiotus* from Costa Rica is described, representing the first record of this genus from Central America.

The Costa Rican fauna of water bears (Tardigrada) is almost completely unknown. Until present only 20 species from 10 genera are known from Costa Rica (Herrera-Vasquez 2003; Herrera-Vasquez & Vargas 2003; Kaczmarek 2003; Kaczmarek & Michalczyk 2004; Michalczyk & Kaczmarek 2006).

Materials and methods

Eight specimens and one egg at the end of the development of *C. longinoi* sp. nov. were found in two moss samples collected in Alajuela Province of Costa Rica. Two specimens were prepared for Scanning Electron Microscope (SEM) and the rest of the specimens were mounted in Hoyer's medium.

All measurements are given in micrometers (μm). Structures were measured only if their orientations were suitable. Body length was measured from the anterior tip to the end of the body, excluding the hind legs. Buccal tube length and level of the stylet support insertion point were measured according to

*Correspondence: Roberto Guidetti, Department of Animal Biology, University of Modena and Reggio Emilia, Via Campi 213/d, I-41100 Modena, Italy. Email: guidetti.roberto@unimore.it

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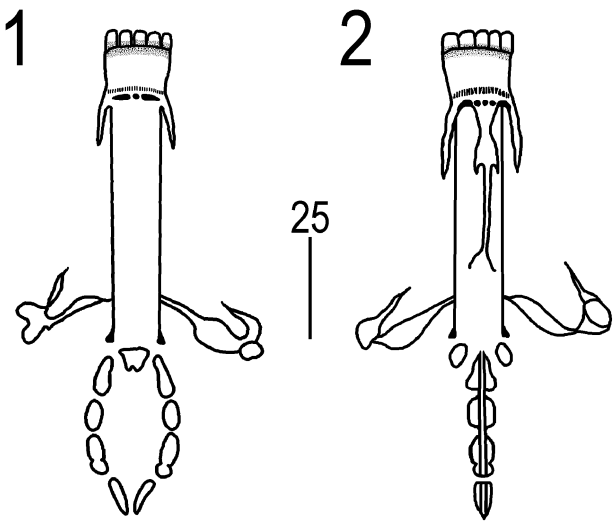


Figures 1 and 2. *Calcarobiotus longinoi* sp. nov. **1–2**: bucco-pharyngeal apparatus (1: dorsal, 2: ventral view).

Pilato (1981). Buccal tube widths were measured as the internal diameters at the level of the stylet support insertion point. Lengths of claws were measured from the base of the claw to the top of the primary/secondary branch, including accessory points. The *pt* ratio is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato 1981). Terminology describing the oral cavity armature is given according to Michalczyk & Kaczmarek (2003).

Photomicrographs and drawings were made using Phase Contrast Microscope (PCM) associated with *camera lucida*, Nomarski Differential Interference Contrast Microscope (DIC) and SEM. LM is an abbreviation for Light Microscopy (DIC and PCM).

Taxonomic accounts

Calcarobiotus longinoi sp. nov.
(Figures 1–20, measurements of holotype are given in Table I)

Material examined: Holotype and 7 paratypes (5 mounted in Hoyer's medium and 2 prepared for SEM) and 1 egg at the end of the development (mounted in Hoyer's medium).

Description

Holotype slide No. CR1079/2. Body transparent/white (Figure 3). Eyes present. Dorsal cuticle with evident granulation on the caudal end of the body (granule 0.5–1.0 in diameter) (Figure 12). Ventral cuticle smooth. Cuticle above claws especially on IV pair of legs with fine granulation (visible in LM and SEM; Figures 17–18). Cuticular pores absent. Bucco-pharyngeal apparatus of *Macrobiotus* type (Figures 1, 2, 4).

Buccal tube with well-developed bend in anterior portion. Mouth antero-ventral with 10 peribuccal lamellae. Oral cavity armature well developed and composed of three bands of teeth. First band with small teeth visible as irregular granules with LM. Second band of teeth in shape of a row of small ridges parallel to the main axis of the buccal tube little longer in central portion (ventral teeth little larger and sometimes dimidiated) (Figures 5–10). Third band of teeth (transversal crests) is divided into two series: ventral and dorsal. Ventral series consists of three to six median teeth and two ridge-shaped lateral teeth (Figures 5–7). Dorsal series consists of two ridge-shaped lateral teeth and one ridge-shaped median tooth (Figures 8, 9). At the end of the buccal tube, three deep double-arched incisions in line with the three placoid rows present but poorly developed. Pharyngeal bulb almost spherical with apophyses, three rod-shaped macroplacoids clearly different in length from each other and very large triangular microplacoid (Figures 1, 2, 4). Apophyses triangular (Figure 1). First macroplacoid clearly narrower in anterior part, second oval, third with subterminal constriction.

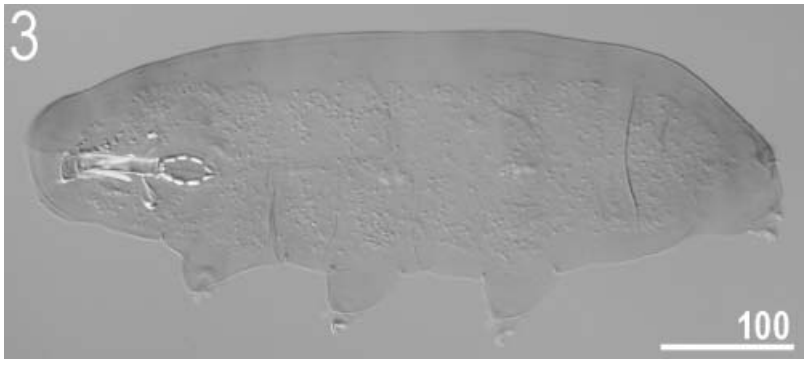
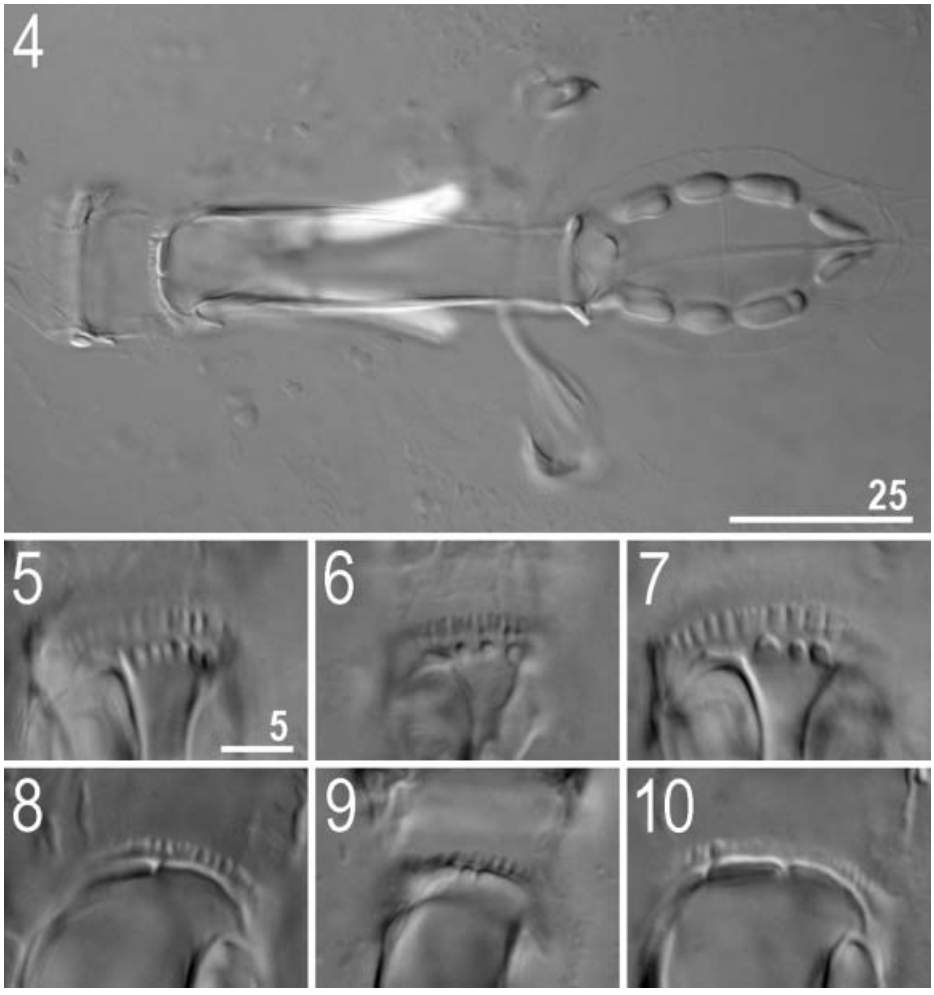
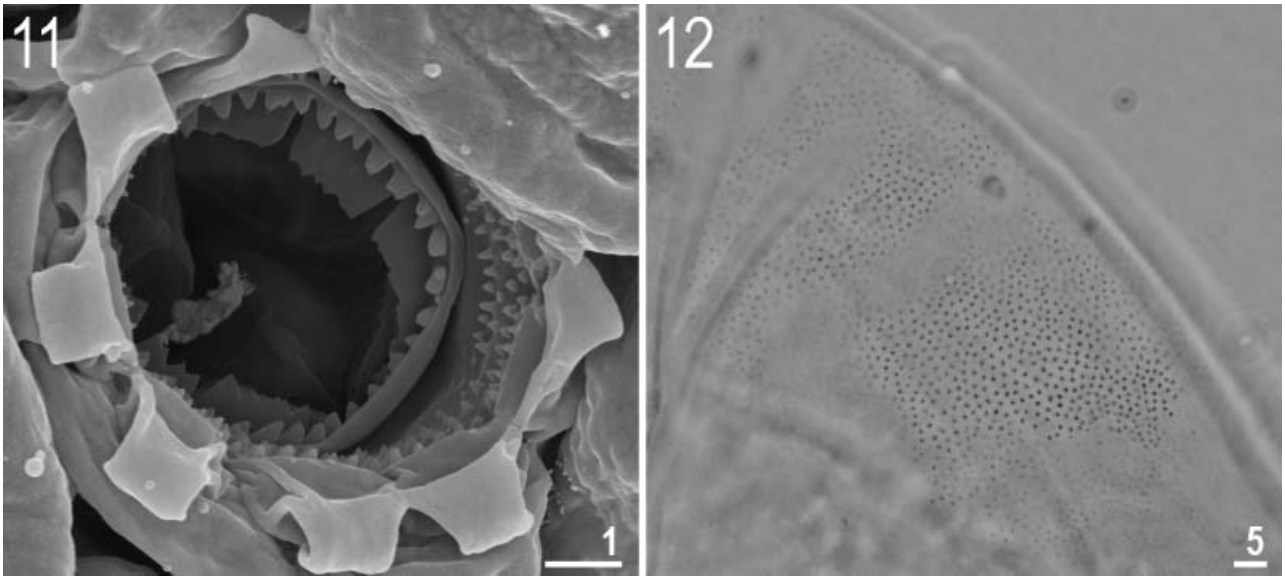


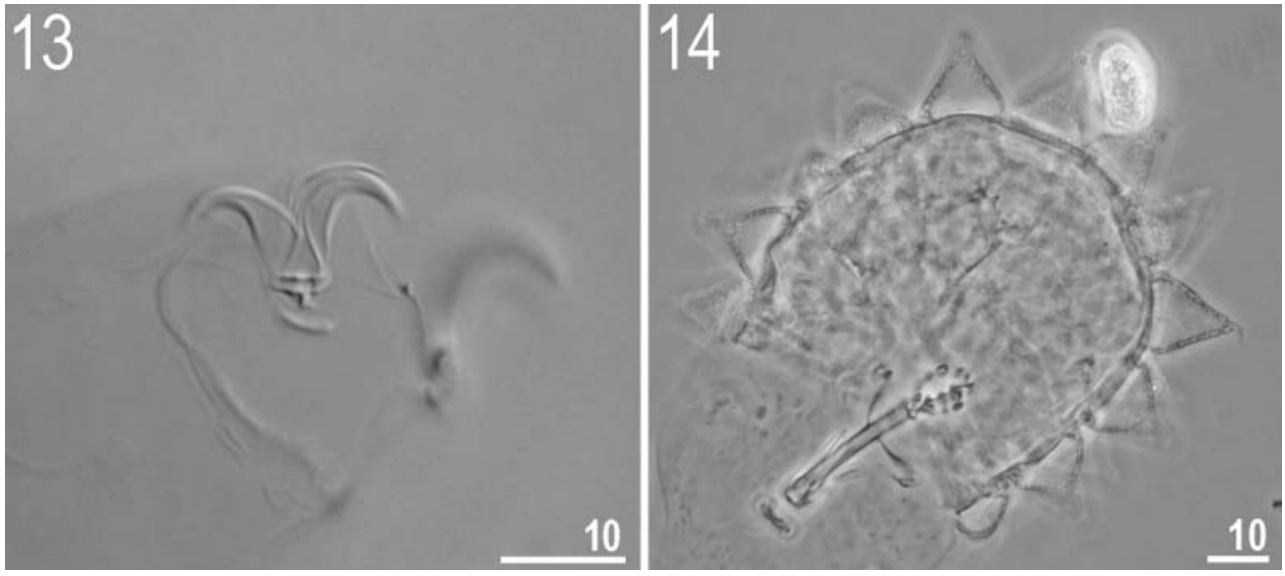
Figure 3. *Calcarobiotus longinoi* sp. nov.: habitus (DIC).



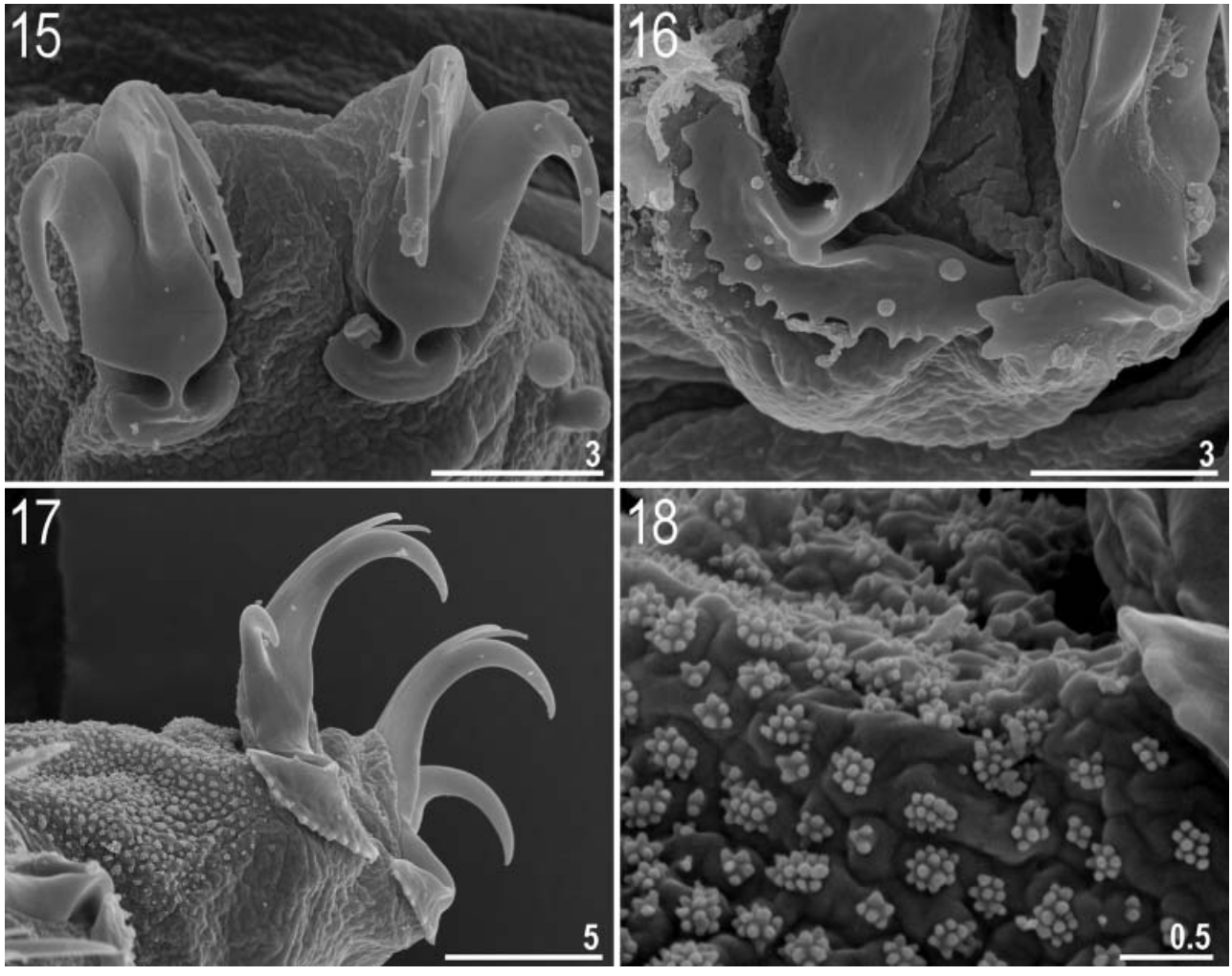
Figures 4–10. *Calcarobiotus longinoi* sp. nov. **4**: bucco-pharyngeal apparatus. **5–7**: oral cavity armature (ventral views). **8–10**: oral cavity armature (dorsal views) (5 and 8, 6 and 9, 7 and 10 come from the same specimens) (DIC, scale on 6–10 the same as on 5).



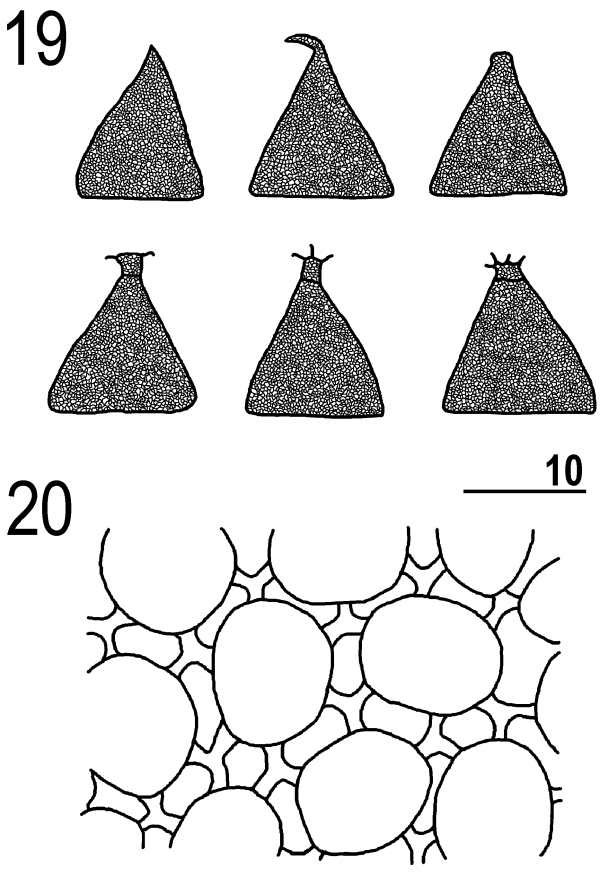
Figures 11 and 12. *Calcarobiotus longinoi* sp. nov. **11**: oral cavity armature (SEM). **12**: granulation on the caudal-dorsal cuticle (PCM).



Figures 13 and 14. *Calcarobiotus longinoi* sp. nov. **13**: claws of the III pair of legs. **14**: embryonated egg (mid-section) (DIC).



Figures 15–18. *Calcarobiotus longinoi* sp. nov. **15**: claws of the I pair of legs. **16**: lunules of claws of the IV pair of legs. **17**–**18**: granulation on the hind legs (Figure **18** shows a magnified area of Figure **17**) (SEM).



Figures 19 and 20. *Calcarobiotus longinoi* sp. nov. **19**: variability of the egg processes (all processes come from one egg). **20**: egg areolation.

Table I. Measurements (in μm) and *pt* values of selected morphological structures of four specimens of *Calcarobiotus longinoi* sp. nov. mounted in Hoyer's medium (in the body length order).

CHARACTER	Paratype 1	Paratype 2	Holotype	Paratype 3
Body	347.7	418.0	532.0	585.0
pt	746.9	758.6	933.3	886.4
Buccal tube	46.6	55.1	57.0	66.0
Stylet support insertion point	38.0	45.6	47.5	55.0
pt	81.6	82.8	83.3	83.3
Buccal tube internal width	7.6	10.0	10.5	12.3
pt	16.3	18.1	18.3	18.6
Macroplacoid 1	5.7	8.6	7.6	8.0
pt	12.2	15.5	13.3	12.1
Macroplacoid 2	4.8	6.7	6.7	7.0
pt	10.2	12.1	11.7	10.6
Macroplacoid 3	6.7	9.5	9.5	12.0
pt	14.3	17.2	16.7	18.2
Microplacoid	6.2	8.1	7.6	10.0
pt	13.3	14.7	13.3	15.2
Macroplacoid row	20.9	26.6	25.7	34.0
pt	44.9	48.3	45.0	51.5
Placoid row	28.5	36.1	35.2	45.0
pt	61.2	65.5	61.7	68.2
Claw 1—external	?	10.5	11.4	13.0
pt	?	19.0	20.0	19.7
Claw 1—internal	?	10.5	10.9	15.0
pt	?	19.0	19.2	22.7
Claw 2—external	?	11.4	13.3	?
pt	?	20.7	23.3	?
Claw 2—internal	?	10.5	12.4	14.0
pt	?	19.0	21.7	21.2
Claw 3—external	?	11.4	13.8	16.0
pt	?	20.7	24.2	24.2
Claw 3—internal	?	11.4	12.4	?
pt	?	20.7	21.7	?
Claw 4—anterior	?	12.4	?	?
pt	?	22.4	?	?
Claw 4—posterior	?	13.3	?	?
pt	?	24.1	?	?

Claws of *Calcarobiotus* type (according to Guidetti & Bertolani 2001), well-developed on all legs (Figures 13, 15). Lateral spurs on claw basal portion absent. Primary branches with distinct accessory points. Lunules smooth on first three pair of legs but distinctly dentate on leg IV (Figures 16, 17). Long transversal cuticular bars present below claws on legs I–III.

Egg: White, spherical, laid freely (Figures 14, 19, 20). Diameter of egg without processes 57.0 and 84.6 including them. Processes (about 16 on circumference of egg) in shape of cones often terminated with few short tips. With LM, the surface of the processes appears reticulated with small irregular meshes. Processes 12.4–15.3 high, basal diameter 11.4–12.4. Egg surface between processes with areolation (usually one areola per one neighbouring process). Surface of areoles smooth.

Remarks and SEM observations

Teeth of first two bands are visible with SEM as small cones which are much higher in the second band (additionally on the ventral side, teeth of the second band are sometimes dimidiated) (Figure 11). Between the first and second band of teeth, cuticular fold is present and situated directly above second band of teeth (Figure 11). This structure was also observed in *Macrobiotus marlenae* Kaczmarek & Michalczyk, 2004 and *Macrobiotus reinhardti* Michalczyk & Kaczmarek, 2004. Teeth in the third band are larger than those in first two bands and visible with SEM as flat, irregularly serrated plates (Figure 11). A high variability was observed in the size, shape and number of teeth in the third band of teeth. On the ventral series, number of median teeth varies from three (in the shape of granules) to six (in shape of small ridges similar to those in the second

band of teeth). On the dorsal series the median tooth may be granule- or ridge-shaped (Figures 5–10).

Granules on IV pair of legs have a complex structure and in SEM they appear as aggregations of small tubercles or cones (2–15 tubercles/cones on each aggregation) (Figures 17, 18).

Type locality

Central America, Costa Rica, Alajuela Province, about 100 m asl, moss from tree near the main road, 6.5 km on south from Upala; 06.01.2003; leg. Ł. Kaczmarek.

Etymology

The name is dedicated in honour of the American zoologist Professor John Longino who helped the first author to prepare the scientific expedition to Costa Rica in 2002/03.

Type depositories

The holotype, 2 paratypes and one embryoned egg (on 1 slide) are deposited in the Natural History Collections, Faculty of Biology, A. Mickiewicz University, Umultowska 89, 61–614 Poznań, Poland. Two paratypes are deposited in the Department of Animal Taxonomy and Ecology, A. Mickiewicz University, Poznań, Poland and 1 paratype is deposited in Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy.

Differential diagnosis

The new species differs from all the other *Calcarobiotus* species by the absence of spurs. Besides, the new species differs from:

1. *C.* (*D.*) *polygonatus* (Binda & Guglielmino, 1991) by the presence of the *Calcarobiotus* claw type on the IV pair of legs and absence of polygonal sculpture on the dorsal side of the body.
2. *C.* (*D.*) *tetrannulatus* Pilato et al., 2004 by the presence of the *Calcarobiotus* claw type on the IV pair of legs, different shape of egg processes and different egg shell design.
3. *C.* (*C.*) *gildae* (Maucci & Durante Pasa, 1980), *C.* (*C.*) *digeronimoi* Pilato et al., 2004, *C.* (*C.*) *filmeri*, *C.* (*C.*) *occultus* and *C.* (*C.*) *imperialis* Abe & Takeda, 2000 by the absence of spur on legs I–III, the presence of fine granulation on the caudal end of the body and different shape of egg processes and different egg shell design.

The key to the species of the genus Calcarobiotus

1. Claws on IV pair of legs different from those of the other pairs (basal tract long and secondary branch short) (subgenus *Discrepunguis*) 2
– Claws on the IV pair of legs of the *Calcarobiotus* type (subgenus *Calcarobiotus*) 3

2. Spur on claws of the first three pair of legs absent, cuticle covered by polygonal sculpture, eyes present ..... *C.* (*D.*) *polygonatus*
– Spur on claws of the first three pair of legs present, cuticle covered by fine dots, eyes absent ..... *C.* (*D.*) *tetrannulatus*

3. Spur on claws of the first three pair of legs present 4
– Spur on claws of the first three pair of legs absent ..... *C.*(*C.*) *longinoi* sp. nov.

4. Spur on the claw basal portion of the IV pair of legs absent 5
– Spur on claws of the IV pair of legs present 6

5. Spurs on claws of legs I–III present on only one side of each claw, eyes present, egg processes reticulated, slender cone-shaped with two or three apices ..... *C.* (*C.*) *gildae*
– Spurs on claws of legs I–III present on both sides of all claws, eyes absent, egg processes smooth, bell-shaped with three apical undulations ..... *C.* (*C.*) *digeronimoi*

6. Eyes present, egg processes in the shape of cones with elongated and flexible tips often ended by 2–3 smaller branches ..... *C.* (*C.*) *filmeri*
– Eyes absent, egg processes bell-shaped 7

7. Egg processes ended with spine like structures ..... *C.* (*C.*) *occultus*
– Egg processes without spine like structures ..... *C.* (*C.*) *imperialis*

Conclusive remarks

The new species has been included into the subgenus *Calcarobiotus* based on the presence of a *Calcarobiotus* claw type on all legs. However, in this subgenus all species have a spur on the basal portion of at least some claws, while in the new species all claws are without spurs. The spurs are also absent on fourth claws in *C* (*C.*) *gildae* and *C.* (*C.*) *digeronimoi*. Guidetti and Bertolani (2001) erected the two subgenera within *Calcarobiotus* due to the identification of two evolutionary lines based on the morphologies of the claws of hind legs. The claws of the hind legs of *Calcarobiotus longinoi* sp. nov. are similar to

those of *C* (*C.*). *gildae* and *C.* (*C.*) *digeronimoi*. Therefore, to include *Calcarobiotus longinoi* sp. nov. in the subgenus *Calcarobiotus*, we emended the definition of the subgenus: Claws with similar shape on all legs. Spur(s) on the basal portion present or absent.

Most of the species of the genus *Calcarobiotus* are known from Africa, Madagascar and the Seychelles (*C.* (*D.*) *polygonatus*, *C.* (*C.*) *occultus*, *C.* (*C.*) *filmeri*, *C* (*D.*) *tetrannulatus*) and three are known from Asia: *C.* (*C.*) *imperialis* (Japan), *C.* (*C.*) *gildae* (Andaman Island) and *C.* (*C.*) *digeronimoi* (Thailand). Seven species including the new species from Costa Rica are known from tropical regions both from the northern and the southern hemisphere. Only one, *C.* (*C.*) *imperialis*, is known from a non-tropical region in Japan. It seems that the genus *Calcarobiotus* is strongly associated with the tropical climate but further research is required to investigate the strength of this relationship.

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